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Introduction

Prostate cancer (PCA) is the most common cancer in men and, second to lung cancer, causes the greatest number of deaths in American males (1,2). While the exact etiology of PCA is largely unknown, it is a multifactorial disease in which several environmental and genetic factors are likely involved (3,4). Epidemiological studies have shown that environmental factors and lifestyle contribute to the development of the disease (5). North Americans and Northern Europeans have the high rates of disease while men in Asian nations experience lower rates (2). Risk is also strongly associated with a family history of the disease, and several susceptibility genes or loci are currently under investigation (6,7). Other etiological factors such as androgens (8), growth factors (9), diet (10-16), sexually transmitted diseases (17), and infection with other infectious agents (18) may contribute to increased susceptibility.

Over the past several decades, a number of epidemiological studies have identified an association between specific bacterial infections and PCA (18). A recent meta-analysis of the literature has demonstrated an increased risk for PCA in men with a history of prostatitis (19). Other investigations of infectious agents in the etiology of chronic prostatitis/chronic pelvic pain syndrome have shown a correlation between the presence of bacterial genes and inflammation in prostate biopsy specimens (20). Chronic or recurrent prostatic inflammation, known to inflict cellular oxidative damage, may thus contribute to the development of PCA.

Chronic inflammation is a significant component in carcinogenesis of the liver, large bowel, urinary bladder, esophagus, and stomach (21,22). A working hypothesis of PCA etiology based on chronic inflammation, reactive oxygen species (ROS)-induced genome damage, and neoplastic transformation has been proposed in the last several years (23). The central concept in this hypothesis is that carcinogenesis results from repeated tissue damage and regeneration in the presence of ROS and reactive nitrogen species (RNS) released from inflammatory cells. In both acute and chronic infections, infiltrating leukocytes and phagocytic cells destroy bacteria by producing ROS and RNS (22); however, these highly reactive molecules also cause oxidative damage to prostatic cellular DNA, proteins, and lipids, leading to genetic mutations, cytotoxicity, and compensatory cell division. It is conceivable that host inflammatory responses during long-term bacterial colonization could provide one source of ROS-producing neutrophils. Reactive molecules would interact with genomic or mitochondrial DNA in normal prostate epithelial cells, causing genomic alterations such as point mutations, deletions, or rearrangements that accumulate and become permanent in the genomes of proliferating cells (24). This sequence may thus provide a mechanism for prostate carcinogenesis (25).

In the inflammation-oxidative stress model, the progression of prostate epithelial cells from normal to neoplastic is marked by defined histologic changes (5,23). The earliest lesion observed is proliferative inflammatory atrophy (PIA) (23,26-29). An important feature of PIA is that the majority of the lesions are associated with long-standing inflammation in the prostate, and many of the proliferating cells have an immature secretory cell phenotype similar to those found in prostatic intraepithelial neoplasia (PIN) and PCA. Thus, this model strongly suggests that PIA may give rise to prostate carcinoma directly or indirectly by development into high grade PIN lesions (23,28-30).

The primary objectives of this project were to use a mouse model of bacterial prostatitis to determine whether chronic inflammation would lead to development of preneoplastic lesions similar to those observed in humans. The key results are presented here.

Body

A. *E. coli* prostatitis studies

One of the objectives on these experiments was to determine some characteristics of an *E. coli* prostatitis in a mouse model based on previous experience with urinary tract infections (UTI) in female mice (31,32). In one study different inbred strains of male mice were inoculated intraurethrally with PBS or 1×10^6 uropathogenic *E. coli* strain 1677 in PBS on day 0 and sacrificed five days later to verify that a prostate infection could be established. Results are shown in Table 1.

Mouse Strain	PBS		<i>E. coli</i>	
	n ¹	CFU ²	n	CFU
BALB/c	7	5	7	16
C57BL/6J	5	7	5	48
C3H/HeJ	6	6	6	5,370
C3H/HeOuJ	6	6	6	138

1. Number of mice per group
2. Geometric mean of *E. coli* colony-forming units/mg tissue

Consistent with results obtained in female mice, BALB/c and C57BL/6J male mice were resistant to urogenital infection, and the two C3H strains developed a urogenital infection; in this case, in the prostate as well as bladder and kidneys. Mice inoculated with PBS alone had essentially no bacteria in the prostate. One implication of these results is that resistance/susceptibility to infection is genetically determined. Infections in C3H/HeOuJ mice were at a lower intensity at this time point than those in C3H/HeJ, but we would expect the localized colonization to increase over time.

We had previously observed that severe *E. coli* UTIs could persist for up to three weeks in female mice from both of these C3H strains and that the C3H/HeJ mice experienced severe morbidity and a majority of these mice succumbed to their infections. Mice from the C3H/HeOuJ strain had bladder and kidney infection intensities equivalent to C3H/HeJ mice at three weeks post inoculation but had a much lower mortality rate from the chronic infections. Therefore, only C3H/HeOuJ male mice were used for experiments investigating the development of preneoplastic lesions in prostates of mice with chronic *E. coli* infections.

B. Prostate histopathology in acute and chronic *E. coli* infection

1. Acute infection

For these experiments, 12-week old, male C3H/HeOuJ mice were inoculated with 2×10^6 *E. coli* 1677 in PBS. The animals received no other treatments and were sacrificed five days later to obtain prostate tissue for histologic evaluation after staining with hematoxylin-eosin (H-E). Both the BALB/c and C3H/HeOuJ mice had cellular infiltrates characteristic of an acute inflammatory response (Figures 1 and 2). Tissue from both mouse strains had neutrophils present; however, some macrophages were also observed in BALB/c prostates. The infiltrate

was present in the interstitial spaces in prostate tissue from both strains of mice. It was interesting to note the presence of inflammatory cells within the gland in C3H/HeOuJ mice but not in BALB/c.



Figure 1. Prostate tissue from a BALB/c mouse five days after intraurethral inoculation with *E. coli*. Inflammatory infiltrate consisting of neutrophils and a smaller proportion of macrophages is present in interstitial spaces. Staining is with hematoxylin-eosin. (100x)

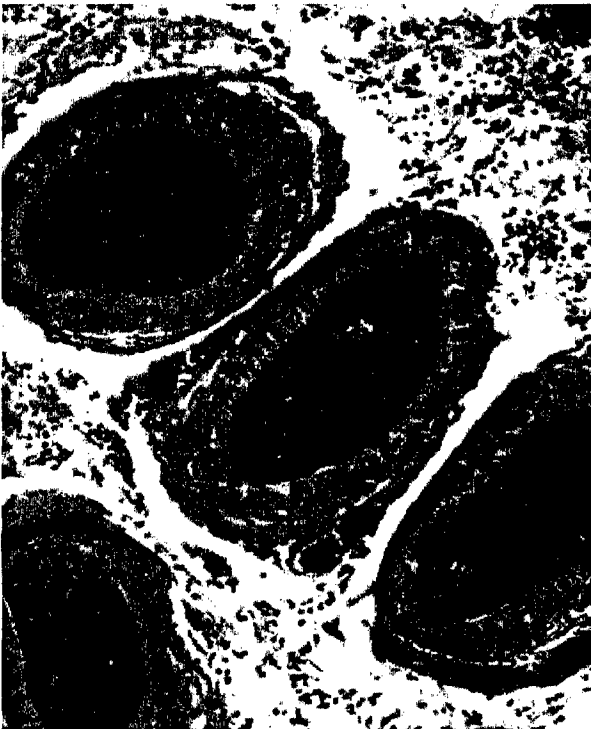


Figure 2. Prostate tissue from a C3H/HeOuJ mouse five days after intraurethral inoculation with *E. coli*. Inflammatory infiltrate consisting of neutrophils is present in interstitial spaces and within the gland. Staining is with hematoxylin-eosin. (100x)

2. Chronic infection

Additional 12 week-old, male C3H/HeOuJ mice were inoculated with 2×10^6 *E. coli* strain 1677, and control mice were inoculated with PBS only. The animals were housed without further treatment for 12 weeks. All mice were sacrificed at this timepoint, and prostate tissue was removed to measure bacterial colonization and for staining with hematoxylin-eosin or by immunohistochemical techniques. The prostate had 9,375 *E. coli*/mg tissue. Prostate tissue obtained from a PBS-inoculated mouse and stained with H-E is shown in Figure 3.



Figure 3. Prostate tissue from a C3H/HeOuJ mouse 12 weeks after intraurethral inoculation with PBS. Note the single layer of epithelial cells and absence of inflammatory cells. Staining is with hematoxylin-eosin. (100x)

Hematoxylin-eosin stains of prostate tissues from C3H/HeOuJ mice inoculated with *E. coli* are shown in Figures 4, 5, and 6 at increasing magnifications. These micrographs show the presence of high-grade PIN in the tissue. Notable features in Figure 4 are evidence of chronic inflammation localized in the stroma and complex glandular proliferation and in the left-most portion of the gland. The right-most portion of the gland shows active, acute inflammation and epithelial cell proliferation. The complex glandular structure of the prostate is also evident in Figure 5. Figure 6 shows histologic features of high-grade PIN lesions in more detail. Here, there is increased layering of the epithelium, papillary structures, and epithelial cell atypia. This atypia is characterized by enlarged nuclei, nuclear polymorphism, and nuclear crowding or overlap. There is also a random orientation of nuclei.



Figure 4. Prostate tissue from a C3H/HeOuJ mouse 12 weeks after intraurethral inoculation with *E. coli*. Tissue shows evidence of acute and chronic inflammation and complex glandular proliferation. Epithelial cell proliferation is also present. Staining is with hematoxylin-eosin. (100x)



Figure 5. Prostate tissue from a C3H/HeOuJ mouse 12 weeks after intraurethral inoculation with *E. coli*. Tissue shows complex glandular proliferation. Staining is with hematoxylin-eosin. (200x)



Figure 6. High-grade PIN in prostate tissue from a C3H/HeOuJ mouse 12 weeks after intraurethral inoculation with *E. coli*. Tissue shows increased layering of the epithelium, papillary structures, and epithelial cell atypia. The atypical features include enlarged nuclei, nuclear polymorphism, nuclear crowding or overlap, and random orientation of nuclei. (400x)

Prostate tissue from C3H/HeOuJ mice taken 12 weeks after inoculation were also stained by immunohistochemical methods using antibodies to detect oxidative damage to DNA (anti-8-OH-2'-deoxyquanosine) or cell proliferation (anti-Ki-67). Anti-8-OH-2'-deoxyquanosine staining of PBS-treated and prostatitis tissue are shown in Figures 7 and 8, respectively. Figure 8 shows background staining of cytoplasm and specific staining of nuclei for the oxidative stress marker.

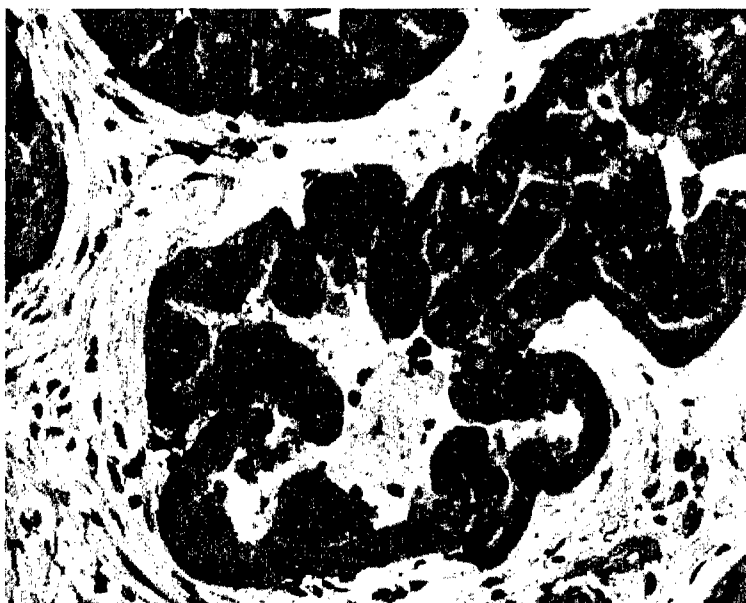


Figure 7. Prostate tissue from a C3H/HeOuJ mouse inoculated with PBS and stained to detect possible oxidative damage to DNA. Immunohistochemical staining was performed with primary antibody to 8-OH-2'-deoxyquanosine and horseradish peroxidase-conjugated secondary antibody to mouse IgG. Counterstaining was with hematoxylin. (400x)

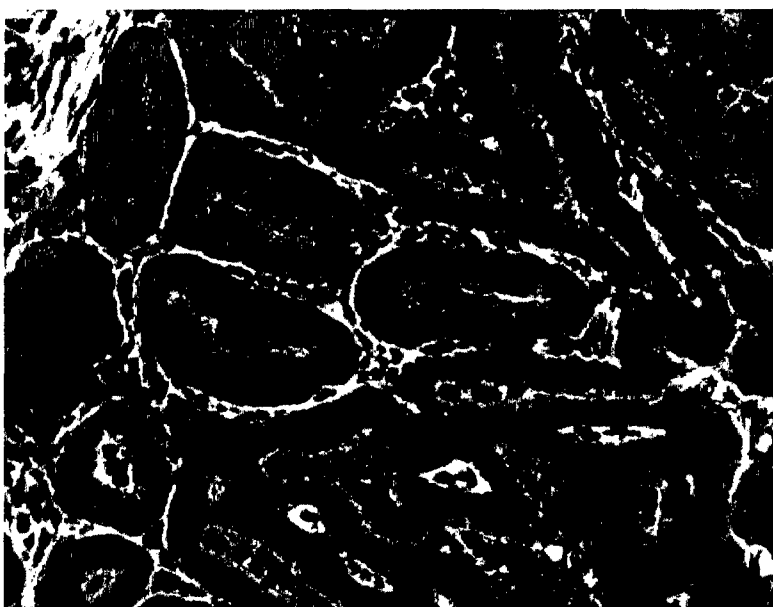


Figure 8. Prostate tissue from a C3H/HeOuJ mouse inoculated with *E. coli* and stained to detect oxidative damage to DNA. Staining method is described in Figure 7. (400x)

Figures 9 and 10 show the results of immunohistochemical staining to detect the cell proliferation marker, Ki-67. Tissue in Figure 9 is from a mouse inoculated with PBS and that in Figure 10 is from a mouse with a chronic *E. coli* prostatitis. There is very little cell proliferation detected in the uninfected tissue, whereas numerous cells stained for Ki-67 in the prostatitis tissue sample.



Figure 9. Prostate tissue from a C3H/HeOuJ mouse inoculated with PBS and stained to detect cell proliferation. Immunohistochemical staining was performed with primary antibody to Ki-67 and horseradish peroxidase-conjugated secondary antibody to rabbit IgG. Counterstaining was with hematoxylin. (400x)

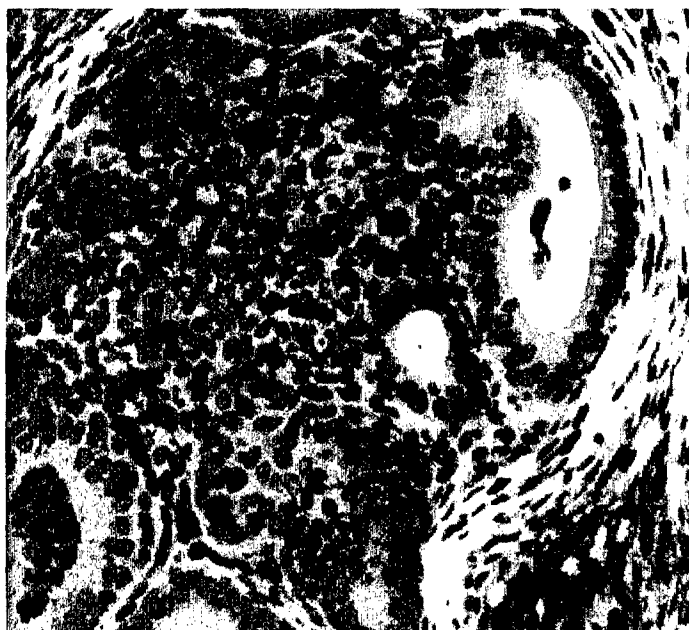


Figure 10. Prostate tissue from a C3H/HeOuJ mouse inoculated with PBS and stained to detect cell proliferation. Staining method is described in Figure 9. (400x)

Key Research Accomplishments

- Application of a mouse model of *E. coli* prostatitis to study the effects of chronic inflammation on cells in the prostate
- Demonstration for the first time that prostatic intraepithelial neoplasia (PIN) could be induced in the mouse following bacterial infection
- Demonstrated that oxidative stress and cell proliferation accompany the development of PIN lesions
- Presented results of these experiments at the 2005 American Association for Cancer Research Annual Meeting

Reportable Outcomes

The primary reportable outcome of this research thus far is presentation of experimental data at the AACR meeting cited above. A manuscript containing is in preparation for submission to a scientific journal. These results can also be used as preliminary data in applying for new funding.

Conclusions

There are two primary conclusions from this work. The first is that a mouse model of long-term *E. coli* infection of the prostate can be used to study the cellular effects of chronic bacterial colonization and inflammation. Two of these effects are oxidative damage to DNA and increased epithelial cell proliferation. The second is that PIN lesions can be induced in mice following intraurethral inoculation with *E. coli*, supporting the concept that localized inflammation and oxidative stress may be important factors in the development of prostatic neoplasia. Thus, the model will be very useful for detailed studies on the time-course of PIN development and the cellular changes taking place in the preneoplastic process.

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Background and objective: Prostate cancer (CaP) is a common cause of morbidity and mortality in men in the developed world. Epidemiological studies have linked chronic inflammation to cancer development in several organs, including the prostate. While the etiology of CaP remains largely unclear, we hypothesized that oxidative stress induced by chronic bacterial infection and inflammation may play a role in pre-neoplastic events within the prostate leading to an initiation and promotion of prostatic carcinogenesis. This hypothesis was tested using a mouse model of *E. coli*-induced prostatitis recently developed in our laboratory.

Methods: Male C3H/HeOuJ mice 12 weeks old were anaesthetized with isoflurane and inoculated intraurethrally with 2×10^6 *E. coli* 1677. Control mice were inoculated with PBS. Mice were sacrificed at five days and 12 weeks after inoculation. Prostate tissue sections were stained with hematoxylin and eosin and were evaluated by light microscopy for inflammatory responses and histologic changes. Also, the presence of oxidative DNA damage and cell proliferation was analyzed by immunohistochemistry using antibodies against 8-hydroxy-2'-deoxyguanosine (8-Oxo-dG) and Ki-67.

Results: All infected mice developed acute prostatitis at five days and 12 weeks after inoculation. Control mice treated with PBS had no prostate infections or inflammation at either timepoint. Prostatic tissue sections showed acute inflammatory infiltrates in the prostate glands and the stroma five days following *E. coli* infection. At 12 weeks, the acute inflammation decreased and focal residual acute inflammation was present only in the prostatic glands. In addition, focal mild to moderate chronic inflammation was present in the stroma. Most significantly, prostatic glandular epithelium showed post-infection atypical hyperplasia with increased epithelial cell layers, cytological atypia, and dysplastic changes of glandular architecture. Some changes mimicked prostatic intraepithelial neoplasia or cancer like-lesions. Immunohistochemical analysis showed that atypical prostate glands had a stronger staining for the oxidative DNA damage marker 8-Oxo-dG than normal and simple hyperplastic glands. The atypical glands also showed a significant increase in epithelial nuclear labeling of the cell proliferation marker Ki-67.

Conclusions: Our study showed that prostatic inflammation results in focal prostatic glandular atypia (e.g., PIN and dysplasia), a potential precursor of prostatic adenocarcinoma. In addition, our results suggest a clear relationship between chronic inflammation and oxidative DNA damage that may play a role in prostate carcinogenesis development. Further studies are needed to define the morphological and immunophenotypical changes within the prostate induced by chronic infection

and to clarify a potential link between inflammation and etiology of prostate cancer.

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